PATENT CLAIMS

1. A modified molecule having the biological activity of human erythropoietin (EPO) and being substantially non-immunogenic or less immunogenic than any non-modified molecule having the same biological activity in an individual when used *in vivo*, wherein (i) the said loss of immunogenicity is achieved by removing one or more T-cell epitopes derived from the originally non-modified molecule and said T-cell epitopes are MHC class II ligands or peptide sequences which show the ability to stimulate or bind T-cells via presentation on class II,
(ii) said modified molecule, when tested as a whole protein in a biological human T-cell proliferation assay, exhibits a stimulation index (SI) smaller than the parental non-modified molecule and smaller than 2.0, and
(iii) said T-cell epitopes to be removed are located on strings of contiguous residues of the originally non-modified EPO molecule, the strings are selected from:
(a) RVLERYLLEAKEAENITTGCAEHCSLNENITVP,
(b) RGQALLVNSSQPWEPLQLHVDKAVSGLRSLTTL,
(c) RTITADTFRKLFRVYSNFLRGKLKLYTGEACRT .

2. A modified EPO molecule according to claim 1, wherein said T-cell epitopes to be removed are located on the following sub-strings of the strings (a) , (b) and (c):
(a1) AKEAENITTGCAEHCSLNENI
(a2) RGQALLVNSSQPWEPLQLHVD;
(a3) TFRKLFRVYSNFLRGKLKLYT.

3. A modified EPO molecule according to claim 1, wherein said T-cell epitopes to be removed are located on the strings as depicted in Table 1.

4. A modified EPO molecule of any of the claims 1 to 3, wherein said T-cell epitopes to be removed are located on 13 to 15 consecutive residues from any of said strings.

5. A modified EPO molecule according to any of the claims 1 to 4, wherein the T-cell epitopes have been removed by substitution of one or more amino acid residues within said strings.

6. A modified molecule having the biological activity of human erythropoietin (EPO) and being substantially non-immunogenic or less immunogenic than any non-modified molecule having the same biological activity in an individual when used *in vivo*, wherein (i) the said loss of immunogenicity is achieved by removing one or more T-cell epitopes derived from the originally non-modified molecule and said T-cell epitopes are MHC class II ligands or peptide sequences which show the ability to stimulate or bind T-cells via presentation on class II, and

(ii) said modified molecule has the amino acid sequence:

APPRLICDSRVLERYLLEAKEAENX^{1}TTGCAEHCSX^{2}NENITVPDTKVNFYAWKRMEVGQQAV
EVWQGLALLSEAVLRGQALLVNSSQPX^{3}EPX^{4}QX^{5}HX^{6}DKAVSGLRSLTTLLRALGAQKEAIS
PPDAASAAPLRTITADTFRKX^{7}X^{8}RX^{9}X^{10}SNX^{11}X^{12}RGKX^{13}KLYTGEACRTGDR

wherein

X^1 = A, G, P ;

X^2 = A, D, E, G, H, K, N, P, Q, R, S, and T;

X^3 = T, A, and G;

X^4 = A, P, D, E, G, H, K, N, P, Q, R, S and T;

X^5 = A, P, D, E, G, H, K, N, P, Q, R, S and T;

X^6 = A, P, D, E, G, H, K, N, P, Q, R, S and T;

X^7 = T;

X^8 = A, P and G;

X^9 = T;

X^{10} = P, A and G;

X^{11} = A, P and G;

X^{12} = S, A, D, E, G, H, K, N, P, Q, R and T;

X^{13} = A, D, E, G, H, K, N, P, Q, R, S and T;

and whereby simultaneously

X^1 = I, X^2 = L, X^3 = W, X^4 = L, X^5 = L, X^6 = V, X^7 = I, X^8 = F, X^9 = V, X^{10} = Y, X^{11} = F, X^{12} = L, and X^{13} = L are excluded.

7. A modified EPO molecule of claim 6, wherein

X^1 = A, X^2 = A, X^3 = T, X^4 = A, X^5 = A, X^6 = A, X^7 = T, X^8 = A, X^9 = T; X^{10} = P, X^{11} = A, X^{12} = S, and X^{13} = A.

8. A modified EPO molecule of claim 5 or 6, wherein the molecule, when tested as a whole protein in a biological T-cell proliferation assay, exhibits a stimulation index (SI) smaller than the parental non-modified molecule and smaller than 2.

9. A DNA molecule coding for a modified EPO protein as specified in any of the claims 1 to 8.

10. A pharmaceutical composition comprising an EPO molecules as specified in any of the claims 1 to 8 together with a pharmaceutically acceptable carrier, diluent or excipient.

11. A peptide sequence being part of a molecule having the biological activity of human erythropoietin (EPO) and comprising one or more T-cell epitopes being MHC class II ligands or sequence tracks which show the ability to stimulate or bind T-cells via presentation on class II; the peptide is selected from the group:

(a) RVLERYLLEAKEAENITTGCAEHCSLNENITVP,

(b) RGQALLVNSSQPWEPLQLHVDKAVSGLRSLTTL,

(c) RTITADTFRKLFRVYSNFLRGKLKLYTGEACRT .

12. A peptide sequence according to claim 11, wherein the sequence is a sub-string of the strings (a) , (b) and (c) and selected from:

(a1) AKEAENITTGCAEHCSLNENI

(a2) RGQALLVNSSQPWEPLQLHVD;

(a3) TFRKLFRVYSNFLRGKLKLYT.

13. A peptide sequence according to claim 11, wherein the sequence is selected from Table 1.

14. A peptide sequence according to any of the claims 11 to 13, comprising 13 to 15 consecutive amino acid residues from any of said strings.

15. A peptide sequence of any of the claims 11 to 14, exhibiting, when tested in a biological human T-cell proliferation assay, a stimulation index (SI) greater than 2.0.

16. A modified peptide sequence of claim 15, wherein the modification results in eliminating potential T-cell epitopes being MHC class II ligands by substitution of one or more

amino acid residues, the peptide exhibits, when tested in a biological human T-cell proliferation assay, a stimulation index (SI) smaller than 2.0, preferably 1.8.

17. Use of a peptide according to claim 16 for the manufacture of a modified human EPO molecule as defined in claim 1.

18. A DNA molecule coding for a peptide sequence as specified in any of the claims 11 to 16.

19. A method of constructing a T-cell epitope map of human EPO by locating T-cell epitopes in human EPO, the method comprising the steps:
(i) in-vitro antigen stimulation using synthetic peptide immunogens using PBMC preparations from unrelated donor samples containing physiologic ratios of T-cell to antigen presenting cells,
(ii) applying computational schemes that simulate the binding of the peptide ligand with one or more MHC allotypes in order to analyse the epitope regions identified in step (i) and thereby identifying MHC class II ligands within the epitope regions;
(iii) applying computational schemes simulating the binding of the peptide ligand with one or more MHC allotypes to identify sequence analogues of the MHC ligands encompassed within the epitope region(s) which no longer bind MHC class II or bind with lowered affinity to a lesser number of MHC allotypes; and optionally
(iv) using naïve T-cell activation assays and synthetic peptides encompassing entirely or in collection encompassing the epitope regions identified within the EPO molecule and testing the sequence analogues in naïve T-cell activation assay in parallel with the parental human EPO sequences.

20. A method of claim 19, wherein the location of a specific T-cell epitope is found when a stimulation index (SI) of 2.0 or greater is observed in at least two independent donor samples.